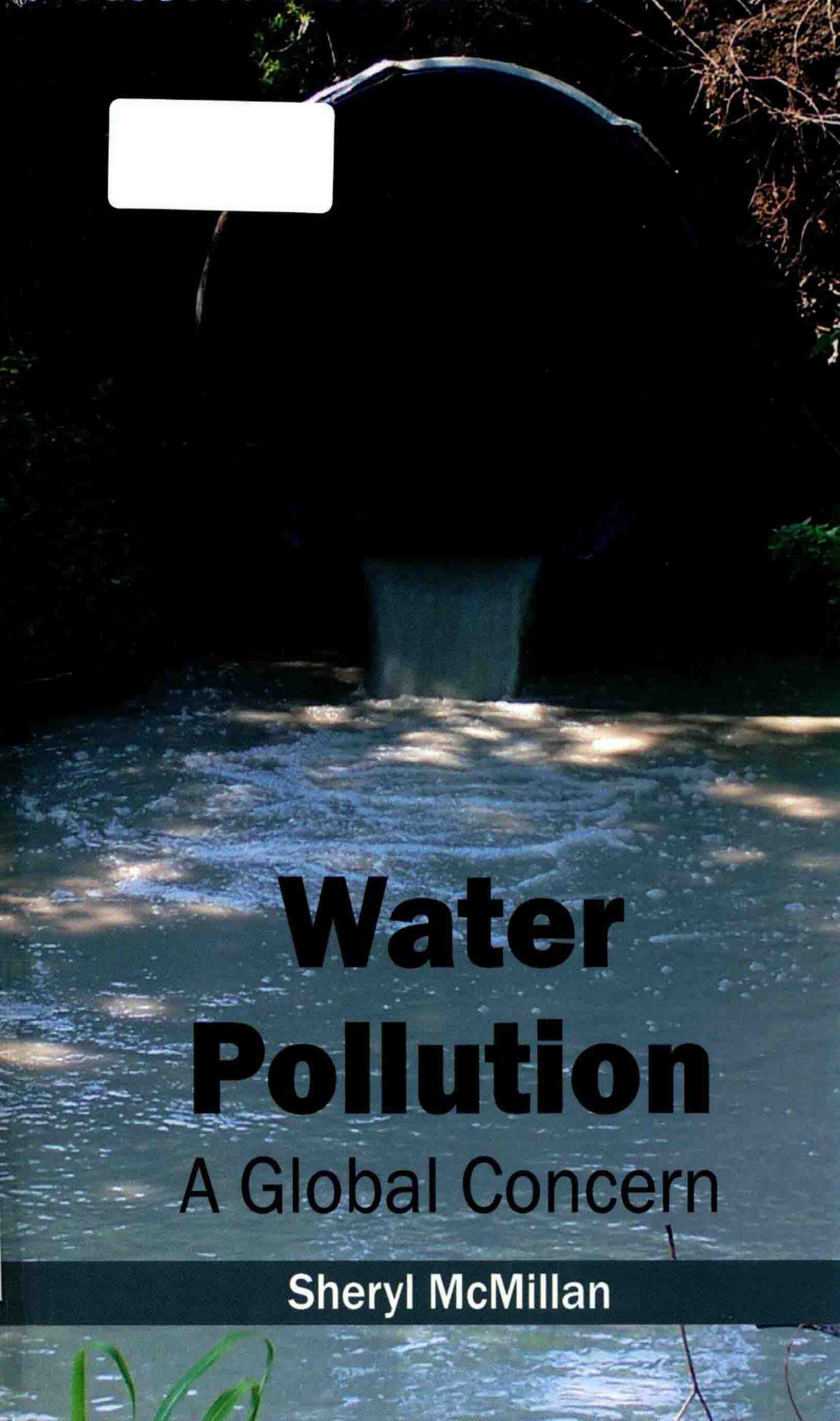

Water Pollution

A Global Concern

Sheryl McMillan

Water Pollution: A Global Concern

Edited by **Sheryl McMillan**

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Preface

The purpose of the book is to provide a glimpse into the dynamics and to present opinions and studies of some of the scientists engaged in the development of new ideas in the field from very different standpoints. This book will prove useful to students and researchers owing to its high content quality.

This book is based entirely on the crucial problem of water pollution. Water pollution is a major issue that needs constant investigation and revision of water resource policy at all levels. It has been implied that it is the leading worldwide cause of deaths and diseases. In addition to the severe water pollution problems in developing countries, industrialized countries keep struggling with pollution problems as well. Water is commonly referred to as polluted when it is contaminated by anthropogenic pollutants and either does not support human usage, such as drinking water, and/or undergoes a marked change in its capacity to support its constituent biotic communities, such as fish. Natural phenomena like volcanoes, algae blooms, storms and earthquakes also cause big changes and shifts in the water quality and the ecological status of water. In the course of time, most water pollutants are carried by rivers into the oceans. This book discusses various facets of water pollution and will be helpful for readers interested in gathering information about this field.

At the end, I would like to appreciate all the efforts made by the authors in completing their chapters professionally. I express my deepest gratitude to all of them for contributing to this book by sharing their valuable works. A special thanks to my family and friends for their constant support in this journey.

Editor

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1

Fluorescence Spectroscopy as a Potential Tool for *In-Situ* Monitoring of Dissolved Organic Matter in Surface Water Systems

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1. Introduction

Water is a common substance, yet life cannot exist without it, being the major component of all living things. Considering the tremendous impact water has on life health, it is always an imperative task to study its quality. During the past decades, more advanced techniques were developed not only to generally characterise the water quality, but also to analyse DOM fractions.

Organic matter is present in every type of aquatic system and, due to the influence that it has on their ecological health, it can be used as a useful water quality indicator. The organic matter fraction from natural waters can be autochthonous, formed in situ through microbial activity, algal productivity, invertebrate grazing, etc., and allochthonous, formed externally and brought into the water system through soil leaching, geological activities or degradation of terrestrial vegetation (Winter et al., 2007). Human activities can influence both of these fractions: increased algal - derived organic matter due to eutrophication increased microbially - derived organic matter from human and animal wastes, and changes in allochthonous organic matter from changes in land use.

An emerging technique, fluorescence spectroscopy, which was successfully used in biology, medicine or chemistry, became a promising approach to the assessment of organic aquatic components and organic pollutants, due to its rapid analysis and high sensitivity. Fluorescence spectroscopy, in the form of three dimensional excitation-emission matrix (EEM), synchronous fluorescence spectrum (SFS) and laser induced fluorescence spectrum (LIFS) can be used to estimate water pollution and to probe the composition of DOM in watersheds. Although the fluorescence technique have been in the attention of those who are interested in real-time monitoring of water pollution, only few studies have been made in this field (Carstea et al., 2010; Downing et al., 2009; Spencer et al., 2007).

This paper proposes to review some of the methods potential to characterise different water systems that have dissimilar hydrological and geographical features and different sources of water pollution. Prior to this, theoretical aspects of fluorescence principles and dissolved organic matter properties will be shortly described.

2. Principles of fluorescence spectroscopy

Fluorescence is a special type of luminescence that describes the emission of light from molecules, named fluorophores, in electronically excited states. The fluorophores absorb energy in the form of light, at a specific wavelength, and release it in the form of emission of light, at a specific higher wavelength (i.e., with lower energy). The general principles of light absorption and emission can be illustrated by a Jablonski diagram, as seen in figure 1.

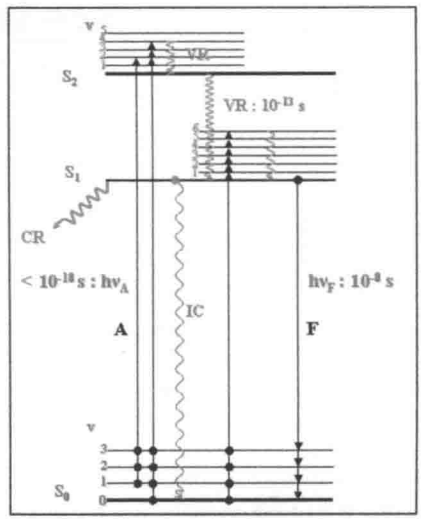


Fig. 1. Jablonski diagram presenting the processes of absorption (A) and fluorescence (F); VR – vibrational relaxation, IC – internal conversion.

When a molecule is found in the ground singlet state, S_0, and absorbs light, the light energy is transferred to the electronically excited states: singlet states, S_1 or S_2. Afterwards, the molecule is subjected to internal conversion or vibrational relaxation, which implies the transition from an upper electronically excited state to a lower one, lasting from 10^{-14} to 10^{-11} s. In the final stage, emission occurs when the molecule returns to the ground state, S_0, in 10^{-9} to 10^{-7} s, emitting light at a greater wavelength, according to the difference in energy between the two electronic states (Lakowitz, 2006; Valeur, 2001). This process is known as fluorescence. When excitation source is a laser, the fluorescence is called laser induced fluorescence.

The Jablonski diagram shows that the energy of the emission is generally less than that of absorption. Thus, fluorescence typically occurs at lower energies or longer wavelengths. This effect is called Stokes' shift, which is caused by several factors: the rapid decay to the lowest vibrational level of S_1, further decay of fluorophores to higher vibrational levels of S_0, solvent effects, excited-state reactions, complex formation, and/or energy transfer (Lakowicz, 2006).

Generally, the emission spectrum for a given fluorophore is a mirror image of the excitation spectrum. The symmetry is a result of the same transitions, which are involved in absorption and emission and the similarities of the vibrational levels of S_0 and S_1 (Christensen, 2005).

2.1 Factors affecting fluorescence intensity

The fluorescence excitation and emission spectra comply with the above-mentioned rule and properties, but several environmental factors can change the characteristics of the fluorescence signal. The fluorescence response is highly affected by solution temperature, composition, concentration, pH and salinity. These factors are presented in the following sections.

2.1.1 Fluorescence quenching

Fluorescence quenching is a term, which covers any process that leads to a decrease in fluorescence intensity of a sample. It is a deactivation of the excited molecule either by intra- or intermolecular interactions. Quenching can be divided into two main categories: static and dynamic quenching.

When the environmental influence (quencher) inhibits the excited state formation, the process is referred to as static quenching. Static quenching is caused by ground state complex formation, where the fluorophore forms non-fluorescent complexes with a quencher molecule. Dynamic quenching or collisional quenching refers to the process when a quencher (e. g. oxygen) interferes with the behaviour of the excited state after its formation. The excited molecule will be deactivated by contact with other molecules or by intermolecular interactions (collision). A wide variety of substances can act as quenchers of fluorescence for different fluorophores (Christensen, 2005; Lakowicz, 2006). In table 1, the quenchers of typical fluorphores are presented.

Typical fluorophore(s)	Quencher(s)
Tryptophan	Acrylamide, halogen anesthetics, hydrogen peroxide, imidazole, histidine, picolinium nicotinamide, succinimide, trifluoroacetamide
Anthracene	Amines, halogens, iodide, thiocyanate
Tyrosine	Disulfides
Polycyclic aromatic hydrocarbons	Nitromethane and nitro compounds
Aromatic hydrocarbons, chlorophyll	Quinones
Naphthalene	Nitroxides, nitric oxide, halogens
Most fluorophores	Oxygen

Table 1. Fluorescence quenchers of typical fluorophores (adapted from Lakowicz, 2006).

Generally, in water the most important fluorescence quencher with high impact on the fluorescence response is temperature. Quenching is enhanced with increasing temperature determining the electrons within a molecule to return to the ground state by a radiationless process. In a study on dissolved organic matter (DOM) thermal fluorescence quenching, Baker (2005) showed that by decreasing the temperature from 45^0 C to 10^0 C the DOM fluorescence intensity increased with ~ 48 %. According to Baker's study (2005) the most affected fluorophore is tryptophan in comparison with fulvic acid.

Fluorescence quenching of dissolved organic matter (DOM) can also be induced using certain metal ions, like Cu^{2+}, Fe^{2+}/Fe^{3+}, Al^{3+}, etc. by the process of complex formation. Metal quenching affects mostly the humic substances and less the amino acids. Most studies have been performed in laboratories, under controlled conditions and little is known about the effects on natural organic matter (Kelton et al., 2007; Reynolds & Ahmad,1995).

2.1.2 Concentration and inner filter effect

Within the context of fluorescence measurements, the inner filtering effect (IFE) represents an apparent decrease in emission quantum yield and/or a distortion of band shape as a result of the absorption and emitted radiation by the sample matrix (Henderson et al, 2009). The fluorescence intensity is attenuated by:

- **Primary inner-filter effect,** referring to the absorption of the excitation beam prior to reaching the interrogation zone;
- **Secondary inner filter effect**, which refers to the absorption of the emitted fluorescence photons (Ohno, 2002);
- **Inner filter effects due to the presence of other substances**. When the solution contains other chromophores that absorb in the same wavelength range as the fluorescent compound under study, the chromophores act as filters at the excitation wavelength and the fluorescence intensity must be multiplied by a correction factor (Valeur, 2001).

In order to be easily understood, the primary and secondary IFE are graphically presented in figure 2.

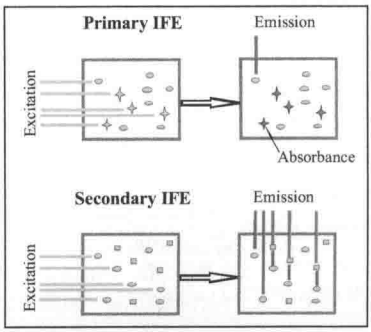


Fig. 2. Diagram of the mechanisms of inner filter effect: primary IFE and secondary IFE.

Various authors have suggested different approaches to correct for IFE, the two most common being an empirical correction based on the Raman scatter peak and a mathematical one based on absorbance profile of the same sample (Parker and Barnes, 1957; Lakowicz, 2006). An alternative approach is to leave the data uncorrected, and utilise the resulting wavelength-dependent non-linear relationship between fluorescence intensity and concentration (Henderson et al., 2009).

The IFE can also be avoided by using front-face illumination because it offers the advantage of being much less sensitive to the excitation inner filter effect. The illuminated surface is better oriented at 30^0 than at 45^0, because at 45^0 the unabsorbed incident light is partially reflected towards the detection system, which may increase the stray light interfering with the fluorescence signal (Valeur, 2001).

Another technique to minimise the IFE is to reduce the path length of the excitation light through the cuvette, but only the primary IFE is reduced. Simple sample dilution to a concentration at which IFE effects are negligible has also been suggested (Baker and Curry, 2004; Baker et al., 2004). There have also been recommendations towards the appropriate concentration quantified as absorbance values. Kubista et al. (1994) suggested that IFE does not occur if the samples show absorbance values lower than 0.05, while Pagano and Kenny (1999) indicate a threshold of 0.01.

2.1.3 Influence of pH on the fluorescence

The pH value of the sample affects the fluorescence of a fluorophore. The pH influence on fluorescence intensity of DOM components always presents the same trend: intensities increase with higher pH until 10, as observed by Reynolds and Ahmad (1995) at raw sewage samples. A small plateau is seen at pH from 5 to 7. Generally, it is not recommended to alter the pH of a sample, but special attention should be paid and the fluorescence spectra should be corrected (Baker, 2007). Similar results have been obtained by Patel-Sorentino et al. (2002) for the humic substances, only that after a fluorescence intensity increase until 10, a slight decrease of its intensity occurs at pH 12.

According to Patel-Sorentino et al. (2002), there are three possible hypotheses:

- **Alteration of the molecular orbital of the excitable electrons**, as a consequence of ionization of the fluorescent molecules after modifications of pH.
- **Macromolecular configuration of humic substances**: the more rigid structures are giving better fluorescent yields. Ghosh and Schnitzer (1980) observed that the structure of humic substances varied with pH changes. Their conclusion is that humic substances have linear structure at high pH and a coil one when pH decreases. Patel-Sorentino et al. (2002) also explained that a spherocolloied configuration could mask some fluorophores inside their structure. At higher pH, the configuration becomes linear and some fluorophores, which are not anymore masked, can fluoresce, increasing the fluorescence intensity.
- **Metal ions present in freshwaters**. This implies that there are some competition phenomena between H^+ ions and metal ions to complex DOM in freshwater, leading to complexation-decomplexation processes which directly affect fluorescence intensity. However, Patel-Sorentino et al. (2002), also note that the metal ions concentration in freshwater samples would be lower than the concentration of metals that quench the fluorescence of DOM. Therefore, the metal ions, which can increase the fluorescence, would have a too weak effect to produce a significant variation in DOM fluorescence intensity.

2.1.4 Salinity influence on the fluorescence

Salinity can affect DOM fluorescence by altering intramolecular reactions, such as conformational change and charge transfer. This results in an increased photoreactivity and fluorescence loss in certain fluorescence compounds (Osburn, 2001; Chen et al., 2002). The relationship between salinity and fluorescence intensity could help detect the source of natural organic matter in marine waters (Elliot, 2006; PhD Thesis).

Del Castillo et al. (1999) investigated changes in chromophoric DOM composition by studying the shifts in the fluorescence maxima. Where the salinity at a site was high, the position of the emission maximum at 350 nm excitation wavelength was shifted to shorter wavelengths, suggesting that high salinity leads to changes in chromophoric DOM.

As shown in previous sections, fluorescence technique is a powerful tool in analysing different samples, but several environmental factors must be taken into account. Most problems arise at highly polluted samples, which imply high concentration of the contaminant and in this case filtration, dilution and absorption to check for IFE are recommended. The pH, salinity and temperature of the sample should be measured, if possible, and the excitation and emission spectra should be corrected, if parameters values are above or under the normal domain. No correction is needed if the sample temperature, at the time of measurement, is between 20^0 C and 25^0 C, or if the pH is between 6 and 8. In

conclusion, the general recommendation is that all these parameters need to be measured before the fluorescence analysis are performed and reported in the scientific literature.

2.2 Techniques for fluorescence spectra recording

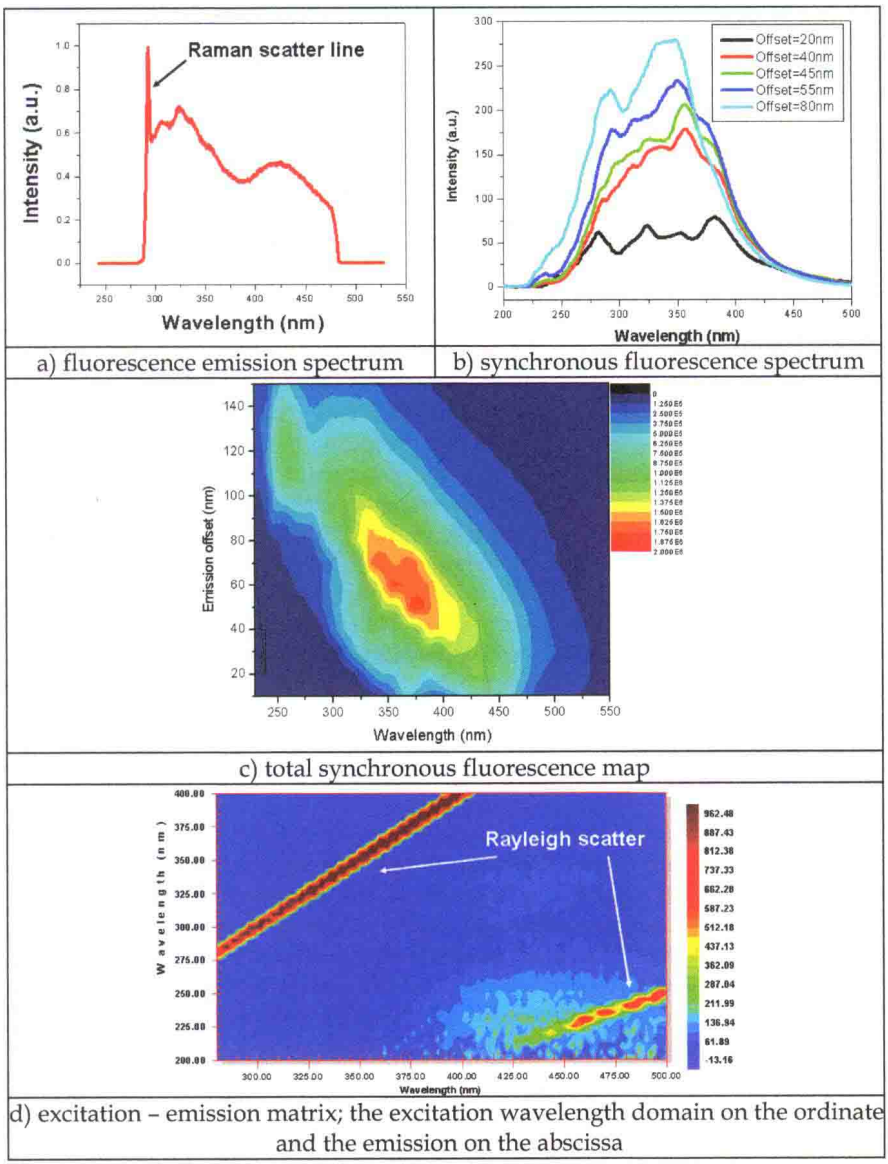
The fluorescence signal is typically recorded as a: fluorescence emission spectrum, fluorescence excitation spectrum, synchronous fluorescence spectrum, total synchronous fluorescence spectrum or excitation – emission spectrum (Figure 3). An emission spectrum consists in the wavelength distribution of the light emission, measured at a single constant excitation wavelength (Figure 3a). Conversely, an excitation spectrum represents the dependence of emission intensity, measured at a single emission wavelength, upon the excitation wavelengths.

In most cases, the analysed samples contain complex multi-component mixtures which cannot be resolved satisfactorily by conventional fluorescence methods. Due to these gaps, for rapid, sensitive, and selective fluorescence analysis, three state-of-the-art methods have been introduced: synchronous fluorescence spectroscopy (SFS), total synchronous fluorescence spectroscopy (TSFS) and excitation-emission matrix (EEM) (Coble, 1996, Deepa and Mishra, 2006; Hudson et al., 2007). As mentioned earlier, an emission or excitation spectrum is recorded by separately scanning the excitation, respectively emission monochromator at various wavelengths. SFS spectra are recorded by scanning both monochromators simultaneously (Deepa and Mishra, 2006) (Figure 3b). Using SFS, the spectral band is narrowed and sharper peaks can be obtained by applying the optimum wavelength offset ($\Delta\lambda$) between excitation and emission. A SFS spectrum is illustrated as fluorescence intensity function of excitation wavelength, for a certain $\Delta\lambda$ (Figure 3c). Total synchronous fluorescence spectrum offers more selectivity and sensitivity to multi-fluorophores mixture analysis. It is presented as a contour map, containing numerous synchronous spectra at different offsets gathered into one bi-dimensional image (Figure 3c). An example of TSFS map is shown in figure 1.18 for a water sample with high protein-like fluorescence intensity.

The last method for complex multi-compounds mixture detection is to record the fluorescence signal as excitation-emission matrices. EEMs represent fluorescence contour maps, in which repeated emission scans are collected at numerous excitation wavelengths providing highly detailed information (Coble, 1996; and references therein) (Figure 3d). Coble (1996) mentions that, once the EEMs have been fully corrected for instrumental configuration, data can be analyzed as excitation spectra, emission spectra or surface spectra, even though originally collected as emission scans (Figure 3d). The EEMs are very simple to analyse because the fluorescence intensity maximum are identified as $\lambda_{excitation}$ / $\lambda_{emission}$ pairs. Usually, the images are colour coded, the highest intensity being represented with red and the lowest with blue.

At a closer inspection of a water fluorescence spectrum, other maxima can be observed. These belong to the scattering of the incident light and are most intense when dealing with turbid solutions and solid opaque samples. Scattering can affect the fluorescence signal, therefore it is of utmost importance to check the absorbance measurements and correct the fluorescence response. Scattered light can be divided into Rayleigh scatter and Raman scatter, according to its nature. Rayleigh scatter is the scattering of light by particles and molecules smaller than the wavelength of the light. Rayleigh scattering represents so-called elastic scatter, meaning that no energy loss is involved, so that the wavelength of the scattered light is the same as that of the incident light. The Rayleigh scatter can be observed

as a diagonal line in fluorescence landscapes for excitation wavelengths equalling the emission wavelengths, as seen in figure 3d. Due to the construction of grating monochromators used for excitation in most spectrofluorometers, also some light at the double wavelength of the chosen excitation will pass through to the sample. For this reason an extra band of Rayleigh scatter, 2nd order Rayleigh, will typically appear in fluorescence measurement (Christensen, 2005).



a) fluorescence emission spectrum

b) synchronous fluorescence spectrum

c) total synchronous fluorescence map

d) excitation – emission matrix; the excitation wavelength domain on the ordinate and the emission on the abscissa

Fig. 3. Typically recorded fluorescence spectra for water samples

Raman scatter is an inelastic scatter, caused by the absorption and re-emission of light coupled with vibrational states. A constant energy loss will appear for Raman scatter, meaning that the scattered light will have a higher wavelength than the excitation light, with a constant difference in wavenumbers. In figure figure 3a, the Raman scattering can be seen as a diagonal line with a systematic, increasing deviation from the Rayleigh scatter line, since the axis is shown as wavelengths, which is not proportional to the energy of the light.
The Raman scatter line can be used to check for instrument stability and to quantify the degree of contamination from a water sample by using the normalised fluorescence intensity to the Raman peak. The advantages offered by the Raman line are: (a) the independence of the chemistry since it measures the properties of the solvent; (b) the ease of application and sensitivity; (c) versatility since it can be applied at any wavelength between 200 and 500 nm. In the case of water the Raman line offers the advantage that it is very stable, appearing in the spectrum at the same offset from the excitation wavelength.

3. Dissolved organic matter fluorescence

The dissolved organic matter (DOM), the ubiquitous fraction in soil and aquatic ecosystems, is a heterogeneous mixture of humic substances, fatty acids and phenolic compounds, amino acids, nucleic acids, carbohydrates, hydrocarbons and other compounds, being among the largest reservoirs of carbon on the planet (Spitzy and Leenheer, 1991; Thomas, 1997; Swietlik and Sikorska, 2004). The dynamics and characteristics of DOM strongly influence a number of key ecosystem processes, including the attenuation of solar radiation, control of nutrient availability, alteration of contaminant toxicity, material and energy cycling (Cammack 2002; PhD Thesis; and references therein). The composition of DOM differs depending on source: it is estimated to contain 0.5 mg/L dissolved organic carbon in alpine streams or 100 mg/L in wetland streams (Spitzy and Leenheer, 1991; Frimmel, 1998). Only 25 % of DOM is fully characterized. It is estimated that 40–70 % from aquatic dissolved organic matter is composed of humic substances (Thurman, 1985; Senesi, 1993).
By the type of production, DOM can be classified as natural or derived from human activity (human wastes, farm wastes, leachates, etc.), but by the origin, DOM can be either allochthonous or autochthonous. Allochthonous DOM, is the fraction that is formed outside the water system and transported inside through discharge, geological and land-use activities or dry and wet deposition (McDowell and Likens, 1988; Hudson et al., 2007). The composition and concentration of allochthonous DOM, in aquatic systems, is dependent mostly on the soil type, catchment, precipitation, vegetation, flow path of water through different soil horizons and other soil processes (Hope et al., 1997; Aitkenhead et al., 1999). Autochthonous DOM is formed within the water system, through derivation from polymerisation and degradation of existing DOM, release from living and dead organisms and through microbial syntheses within the body water (Thomas, 1997).
Within the complex heterogeneous mixture of DOM, only the following components are mostly studied by fluorescence: proteins and humic substances. The protein fluorescence is given by the amino acids tryptophan, tyrosine and phenylalanine, and is related to the activity of bacterial communities, as shown by Cammack et al. (2004) and Elliot et al. (2006a). The humic substances fluorescence indicates the break-down of plant material by biological and chemical processes in the terrestrial and aquatic environments (Elkins and Nelson, 2001; Stedmon et al., 2003; Patel-Sorrentino et al., 2004). Humic substances are divided into two major fractions, depending on the solubility at different pH values: humic

acids which are insoluble in aqueous solution at pH lower than 2, but soluble at higher pH and fulvic acids soluble in water under all pH conditions (Aiken et al., 1985). DOM fluorophores are schematically represented in figure 4, along with their corresponding excitation/emission wavelengths domains. Due to the difficulties associated with identifying of the individual fluorescent compounds in waters, these groups of fluorophores are commonly named humic-like, fulvic-like and protein-like (specifically tryptophan- or tyrosine-like), so called because their fluorescence occurs in the same area of optical space as the standards of these materials (Hudson et al., 2007).

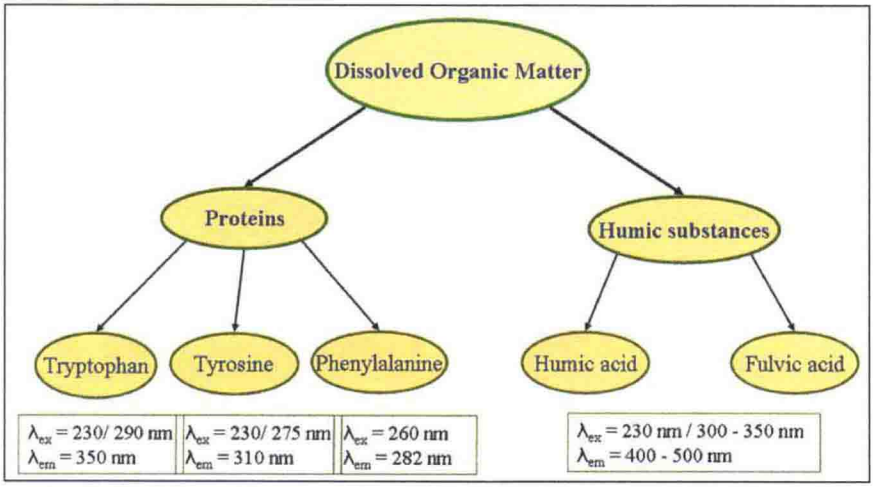


Fig. 4. Schematic representation of DOM fluorescent fractions with the specific excitation/emission wavelengths domains.

Beside standard nomenclature (e.g. humic-like), Coble (1996) defined the humic substances as peak **A** ($\lambda_{excitation}$ = 230 nm, $\lambda_{emission}$ = 400 – 500 nm) and peak **C** ($\lambda_{excitation}$ = 300 – 350 nm, $\lambda_{emission}$ = 400 – 500 nm), tryptophan as peak **T** and tyrosine as peak **B**. Tryptophan, also, presents two excitation wavelengths, therefore, $\mathbf{T_1}$ corresponds to the peak at $\lambda_{excitation}$ = 290 nm and $\mathbf{T_2}$ to the peak at $\lambda_{excitation}$ = 230 nm (Figure 5).

Certain types of contaminants and their relative impact on the system can only be determined by analysing the fluorescence intensity, excitation and emission wavelengths of the above mentioned fluorophores. In the past decades, numerous studies have shown that these fluorophores can provide more information about the characteristics of DOM and the aquatic system. According to some studies, peak C fluorescence intensity correlates with total organic carbon (Smart et al., 1976; Vodacek et al., 1995; Ferrari et al., 1996) and shows a linear relationship with aromaticity (McKnight et al., 2001). Fluorescence intensity of peak C also relates with the molecular weight of the organic fractions, showing lower values for smaller molecular weight fractions (Stewart and Wetzel, 1980). Peak C emission wavelength shows the degree of hydrophobicity, a higher emission wavelength corresponding to greater degree of hydrophobicity (Wu et al., 2003). Peak T presents a very strong correlation with the standard parameter biological oxygen demand (BOD). Some researchers (Reynolds and Ahmad, 1999; Hudson et al., 2008) even tested the possibility of using peak T fluorescence as a surrogate for the standard water quality parameter, BOD. The relationship between

biological activity of aquatic plankton, along with algae metabolism rates, and peak T fluorescence intensity for different DOM types has also been observed (Bieroza et al., 2009 and references therein).

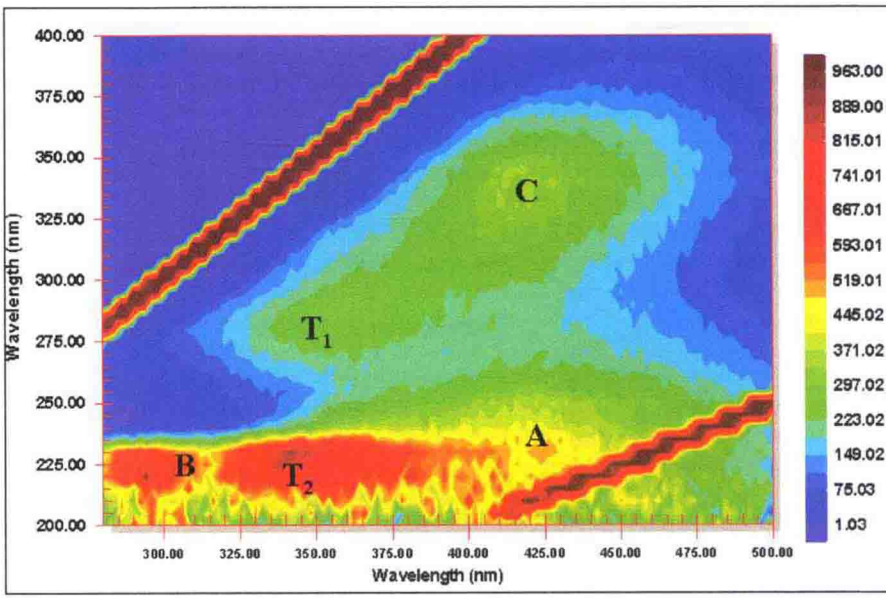


Fig. 5. Excitation – emission matrix presenting the fluorescence domains of the humic – like, peaks A and C, and protein-like fractions, peak B (tyrosine) and peaks T_1 and T_2 (tryptophan).

4. Fluorescence properties of common aquatic pollutants

Water systems naturally contain, as shown in previous sections, organic matter with two very important fluorescence components: humic substances and proteins, which differ in quantity depending on the water body. When high quantities of one component is produced or released into the water, then the balance of the ecosystem is disrupted with potentially long-term effects. Apart from humic substances and proteins, there are other compounds that can contaminate the water and can be detected with fluorescence spectroscopy: polycyclic aromatic hydrocarbons (PAHs), pesticides, environmental hormones. Pesticides and PAHs reach the aquatic environment through direct runoff, leaching, careless disposal of empty containers, equipment washing a.s.o. (Konstantinou et al., 2006). Due to their toxic nature, persistence in the environment and presence in any type of system (soil, surface water, groundwater) many studies concentrated on sensitive, selective and early detection of these pollutants (e.g. Ferrer et al., 1998; Jiji et al., 1999; Jiji et al., 2000; Selli et al., 2004; Deepa et al., 2008). The standard techniques for pesticides and PAHs detection are gas and liquid chromatography, which require tedious extraction or separations procedures and expensive equipments. Fluorescence spectroscopy is a rapid and cost effective alternative, since PAHs and many pesticides are naturally fluorescent (Jiji et al., 1999). When dealing with more components, the use of SFS or EEM techniques is recommended.